Phenolic content, antioxidant potential, and antimicrobial activities of fruit and vegetable by-product extracts

UNIVERSITÀ DEGLI STUDI DI TORINO

PHENOLIC CONTENT, ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITIES OF FRUIT AND VEGETABLE BY-PRODUCT EXTRACTS

Asma Agourram[1], Daniela Ghirardello[2], Kalliopi Rantsiou[2], Giuseppe Zeppa[2], Simona Belviso[2], Abderrahmane Romane[1], Khalid Oufdou[3], Manuela Giordano[2*]

[1]Laboratory of Applied Organic Chemistry, University of Cadi Ayyad, Marrakesh, Bd Prince My Abdallah, 40000, Morocco.

[2]DIVAPRA, Faculty of Agriculture**,** University of Turin, Grugliasco (TO), Via L. da Vinci 44, 10095, Italy.

[3]Laboratory of Biology and Biotechnology of Micro-organisms**,** University of Cadi Ayyad, Marrakesh, Bd Prince My Abdallah, 40000, Morocco.

Address correspondence to Manuela Giordano, DIVAPRA, Faculty of Agriculture**,** University of Turin, Grugliasco (TO), Via L. da Vinci 44, 10095, Italy. E-mail: manuela.giordano@unito.it

ABSTRACT

The use of fruit and vegetable by-products as natural food additives has recently been suggested, due to their richness in polyphenols. The aim of this research study was to determine polyphenolic content and the antioxidative and antimicrobial activities of thirteen fruit and vegetable by-product extracts obtained with three solvent mixtures. The Folin-Ciocalteu method was employed to calculate the total phenolic content (TPC) while antioxidant capacity (AC) was assessed with $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. The highest TPC and AC values were obtained for the acetonic extracts. Pomegranate peels and hazelnut skins showed the highest values of TPC (212.3 and 166.3 mg GAE/g dw respectively) and AC (95.7 and 92.9 of inhibition percentage respectively for $DPPH^{\bullet}$ assay). The antimicrobial activity against twelve foodborne pathogens and spoilage microorganisms was evaluated. Pomegranate and apple peels showed the highest inhibition of *Staphylococcus aureus* and *Pseudomonas fluorescens*. The results obtained demonstrated that by-products could be used as natural food additives with beneficial health properties.

Keywords: by-products, antioxidant capacity, antibacterial activity, DPPH, total phenols

Running title : Antioxidant activities of by-products

INTRODUCTION

Polyphenols are a very important part of our everyday diet, since they are naturally present in fruit and vegetables. As free radical scavengers, they can potentially interact with biological systems and play a role in preventing human neurodegenerative diseases and cardiovascular disorders.[1,2] Besides having a strong antioxidant effect,[3,4] polyphenols often also exhibit antimicrobial activity.[5]

A large number of plants have been examined to define their polyphenolic content and profile.[6-14]

Recently, polyphenolic content was also examined in some plant by-products[15,16] which are available in large quantities and at low cost[17] but are currently used only as feedstuffs or fertilizers. Their use as food additives could help industries to solve the environmental problems related to the disposal of these materials,[18] and provide new sources of natural antioxidants.[19] Thus, the aim of this research study was to determine the polyphenolic content and related antioxidative and antimicrobial properties of extracts obtained from thirteen fruit and vegetable by-products produced in Italy (pomaces from pomegranate, apple, white grape and red grape; peels from pomegranate, apple, hazelnut, white potato and purple potato; seeds from dog rose and cornelian cherry; leaves from leek). An ultrasound-assisted liquid-solid extraction procedure with three different solvent mixtures (methanol/water/acetic acid, ethanol/water and acetone/water) was employed. The total phenolic content (TPC) was evaluated by the Folin-Ciocalteau method, while the antioxidant capacity (AC) was assessed by means of two *in vitro* assays, the DPPH radical scavenging assay (RSA) and the ABTS or TEAC (Trolox equivalent antioxidant capacity) assay. Antimicrobial activity was screened by the agar-well diffusion method, using twelve different foodborne pathogens and spoilage microorganisms.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, (+)-catechin hydrate, gallic acid and HPLC grade methanol were purchased from Sigma-Aldrich (Milan, Italy). HPLC grade acetone and ethanol, 2,2'-azino-bis-(3-ethylbenzothiazolin-6-sulfonate) diammonium salt (ABTS), sodium carbonate and Folin-Ciocalteu reagent were purchased from Fluka (Milan, Italy). Acetone Brain Heart Infusion Broth and technical agar (Agar No. 3) were bought from Oxoid (Milan, Italy).

Plant material collection and extraction

The thirteen by-products listed in Table 1 were purchased directly from producers in Piedmont (North-West Italy). For pomegranate, pomace was used, i.e. the solid remains left over after crushing arils for juice extraction. Pomace contains the pulp and seeds of the fruit. The peel was examined separately. For apple, both the pomace and the peel were examined. The "Grigia di Torriana" apple variety, a typical apple produced in Piedmont, was examined. This variety is generally used for juice and jam production, and is characterized by brown peel and high astringency. Potatoes are also typical of North Italy. The "Viola" (purple) potato is a typical cultivar, also known in France as "Violette noir" or "Truffle potato". A sample of the "Nocciola Piemonte PGI" hazelnut kernels, namely "Tonda gentile Trilobata" *cultivar* was collected. Shortly prior to analysis, hazelnut skins were removed by roasting at 160°C for 20 min in a drying ventilated oven (Mazzali Moduvers, Monza, Italy). For dog rose, cornelian cherry and potato, the pulp was also examined.

Fresh samples were washed, frozen in liquid nitrogen and lyophilized (LIO-5P, Cinquepascal, Milan, Italy), while dried samples were simply ground by a high-speed mill (IKA A11 Basic, Germany). Each sample (1 g) was extracted with 50 ml of three solvent mixtures: methanol/water/acetic acid (90:9.5:0.5, v/v/v); ethanol/water (80:20, v/v) and acetone/water (70:30; v/v). Extraction was performed in darkness, by ultrasound bath (Bransonic® 220, Sigma-Aldrich, Milan, Italy) working at 48 kHz for 15 min at 20 °C. The extracts were centrifuged at 3500 rpm for 20 min. The supernatant was collected, stored at + 4°C and the sample was re-extracted twice using the same procedure. Finally, the extracts were combined, filtered through 0.45 µm filters (Sartorius Stedim Biotech, Florence, Italy) and used to evaluate the total phenolic content (TPC) and antioxidant capacity (AC). An aliquot of the extracts was concentrated to dryness by rotary evaporation at 35 °C under reduced pressure (Büchi Rotavapor® R-210, Flawil, Switzerland). The solid residue was

dissolved in distilled water and lyophilised. Powders thus obtained, (maintained in darkness and nitrogen atmosphere), were used in the antimicrobial activity evaluation.

Total phenols assay

The total phenolic content (TPC) of the extracts was evaluated by the Folin-Ciocalteu colorimetric method.[20] Briefly, 500 µl of extract, or gallic acid standard solutions, and 2.5 ml of 1:10 diluted Folin-Ciocalteu phenol reagent were mixed in a 10 ml test tube. After exactly 3 min, 2 ml of 7.5 % (w/v) aqueous sodium carbonate were added, the mixture was mixed again and then left to stand at 45 °C in the dark for 15 min. The absorbance, against appropriate reagent blank, was read at 765 nm in disposable 1 cm path length polystyrene (PS) cuvettes (VWR International, Milan, Italy) with a UV-1700 Spectrophotometer (PharmaSpec, Shimadzu, Milan, Italy). Gallic acid standard solutions were prepared by dissolving gallic acid in water at concentrations ranging from 0 to 250 mg/L. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of dry extract (dw). All samples were analyzed in triplicate.

DPPH radical-scavenging assay (RSA)

Free radical-scavenging ability of fruit, vegetable and by-product extracts was based on the reaction with the stable radical DPPH, in accordance with the procedure outlined by von Gadow et al.[21] In a 5 ml test tube, 75 µl aliquot of extract was added to 3 ml of DPPH$^{\bullet}$ methanol solution (6.1×10^{-5} M). The mixture was mixed and left to stand at room temperature in the dark for 60 min. The absorbance was read spectrophotometrically at 515 nm in disposable PS cuvettes (1 cm path length) against a control methanol solution of DPPH$^{\bullet}$. The inhibition percentage (IP) of DPPH$^{\bullet}$ was calculated according to the following equation:

$$IP[\%] = (A_{control} - A_{sample})/A_{control} \times 100$$

where A_{sample} and $A_{control}$ are the absorbance values of the reaction mixture with and without samples, respectively. All samples were analyzed in triplicate.

TEAC assay

The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of the ABTS radical cation by antioxidants, was performed according to the modified method of Re et al.[22] The pre-formed radical monocation of 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) was generated by oxidation of ABTS aqueous solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$), and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Just before analysis, the resulting blue-green $ABTS^{\bullet+}$ stock solution was diluted with ethanol to an absorbance of 0.700 (± 0.020) at 734 nm and equilibrated at 30 °C in the dark. A reagent blank reading was taken (A_{blank}). In a 5 ml test tube, 30 µl of extracts were added to 3 ml of diluted $ABTS^{\bullet+}$ solution. The extinction at 734 nm (1 cm path length PS cuvettes, 30 °C) was measured exactly 6 min after the initial mixing. The $ABTS^{\bullet+}$ scavenging effect (% Inhibition) was calculated as follow:

$$\% \text{ Inhibition} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

where A_{blank} and A_{sample} are the absorbances of $ABTS^{\bullet+}$ working solution before and after the sample addition. A calibration curve was prepared with different concentrations of Trolox (0-350 µM/l) and the antioxidant capacity was expressed as µM of Trolox equivalent (TE) per g of dry extract (dw). All samples were analyzed in triplicate.

Antibacterial assay

An overnight culture of approximately 10^8 colony forming units (CFU)/ml was used for all the microorganisms. A range of microorganisms were used as indicators: *Listeria monocytogenes* NCTC 10527, *Staphylococcus aureus* ATCC12606, *Bacillus cereus* DSM 350, *Lactobacillus*

sakei DSMZ 6333, *Lactococcus lactis* DSM 4366, *Staphylococcus xylosus*, *Salmonella*, *Pseudomonas fluorescens*, *Escherichia coli DH5α*, *Escherichia coli* ATCC 35150 (Shiga toxin-producing *Escherichia coli*), *Pseudomonas aeruginosa* and *Serratia marcescens*, from the collection of the Department of Exploitation and Protection of the Agricultural and Forestry Resources, University of Turin, Italy. Strains that did not originate from an international culture collection, were isolated from foodstuffs and their identification to the species level was performed by 16S rDNA sequencing. The agar-well diffusion method was used to determine antibacterial activity.[23] Sterile BHI (Brain Heart Infusion) agar was mixed with the indicator microorganism (final concentration 1% v/v) and poured into sterile standard Petri dishes (20 ml). After setting, medium cups of 6 mm diameter were prepared. For each test, 70 µl of the different solutions with 10 and 20 mg of extract/ml concentration were added to the well.[5] Pure methanol was used as control. After incubation at 37 °C for 24 h, the resulting inhibition zone diameters were measured. All tests were performed in triplicate.

Statistical analysis

Data, unless otherwise specified, were expressed as mean ± standard deviation of triplicate experiments. Statistical analysis was performed with the SPSS software package (version 12.0 for Windows, SPSS Inc., Chicago, Illinois). The one-way analysis of variance (ANOVA) with Duncan's test was carried out to compare samples. The relationship amongst TPC and AC assays was described by the Pearson correlation coefficient *r*.

RESULTS AND DISCUSSION

Total phenolic content

The total phenolic content (TPC) of fruit and vegetable by-product extracts obtained using three

different solvent mixtures are reported in Table 2. Results showed that solvents had significantly different capacities in the extraction of polyphenols, and the most effective was the aqueous acetone solution. Taking into consideration only those extracts obtained with this solvent, the TPC ranged from 212.3 mg of GAE/g dw in pomegranate peel to 1.6 mg GAE/g dw in "Piatlina" potato pulp. The TPC of extracts could be subdivided into three groups, namely high (≥ 50 mg GAE/g dw), medium (< 50 − ≥ 20 mg GAE/g dw) and low (<20 mg GAE/g dw). Pomegranate, hazelnut and apple skin, dog rose pulp, white marcs and cornelian cherry pulp all belong to the first group. Cornelian cherry seeds, red marcs and dog rose seeds belong to the second group, while potato peel and pulp, leek leaves, pomegranate and apple pomace pertain to the third.

The TPC of pomegranate peel aqueous acetone extracts (212.3 mg GAE/g dw) was nearly 16-fold higher than that of aril pomace extracts (13.2 mg GAE/g dw), according to data reported by Li et al.[24] where larger amounts of phenols were found in pomegranate peel with respect to arils (249.4 mg GAE/g and 24.4 mg GAE/g dry extract respectively). Nasr et al.[25] determined a similar TPC content (216.9 mg GAE/g dw) in pomegranate peel extract while, more recently, Vijaya Kumar Reddy et al.[13] reported a TPC of 2.2 mg GAE/g fresh weight for acidified aqueous methanol extract of pomegranate arils. Besides pomegranate peel, roasted hazelnut skin could also be considered a polyphenol-rich product, showing 166.3 mg GAE/g dw of TPC for aqueous acetone solvent. Contini et al.[26] reported a TPC of 466.8 mg GAE/g dw from skin waste of whole roasted hazelnut, but extraction figures differed with long maceration time of the defatted product.

The TPC of dog rose pulp aqueous acetone extracts was similar (85.5 mg GAE/g dw) to those reported by Wenzig et al. (82.2 mg GAE /g dw).[27] Our study also analysed the dog-rose seeds, a by-product obtained during jam production. Extracts of dog-rose seeds showed a low value of TPC (21.5 mg GAE/g dw). This value is lower than that of fruits but interesting when considering the use of seeds as a low-cost additive for functional foods. The TPC of cornelian cherry pulp fruits (50.1 mg GAE/g dw) was higher than that reported by Ju and Hsieh (20.9 – 33.4 mg GAE/g semi-dried fruits),[28] Marinova et al. (4.3 mg GAE/g fresh mass),[29] and Pantelidis et al. (15.9 mg GAE/g

dw)[30] but comparable with data published by Yilmaz et al. (26.6 – 74.8 mg GAE/g dw).[10] The TPC of cornelian cherry seeds (37.7 mg GAE/g dw) was higher than that of dog rose seeds and similar to that of fruits.

Apple fruits have been widely investigated as a good source of polyphenols. Suarez et al. [31] reported apple pomace to have a higher value for acetonic extracts (6.5 g GAE/kg dw) than for methanolic extracts (3.6 g GAE/kg dw). Wolfe et al. [32] showed that extracts of apple peel exhibited significantly higher TPC than those of apple pulp. Similar results were obtained in this study with 61.3 mg GAE/g dw for apple peel and only 10.4 mg GAE/g dw for apple pulp. .

Among vegetables, the TPC of acetonic extracts of potato peel and pulp from different Italian varieties ("Viola", "Desirée" and "Piatlina") showed very low values. The purple "Viola" variety, exhibited the highest TPC among potato samples (8.9 mg GAE/g dw). Al-Weshahy and Rao reported that the TPC of peel for six varieties ranged from 1.5 to 2.1 mg GAE/ g dw for black tuber and from 2.9 to 3.3 mg GAE/g dw for red tuber. [33] The differences in values of TPC were probably due to the colour and variety of potato tested [34] but also a result of the presence of anthocyanins in the skin of colored potato varieties, which was found to be 2.5- fold higher than in the tuber pulp.[35]

The TPC of leek leaf aqueous acetone extract was 7.0 mg GAE/g dw. Few studies are available on polyphenolic content of leek and even fewer devoted to the edible part of this vegetable. Marinova et al.[29] and Dragović-Uzelac et al.[36] reported a TPC of 35.7 and 75.3 mg GAE/ 100 g fresh mass respectively but, in the absence of data about dry matter, these values are not comparable with those obtained in this study.

Data published by Turkmen et al. [37] was more comparable as they reported a TPC of 3.0 mg GAE/g dw for leek. As the edible part of leek is formed by modified leaves, it is possible that its polyphenolic composition is similar to that of non-modified leaves which are by-products. Regarding marc extracts, the TPC for marc obtained from white grapes was significantly higher (50.5 mg GAE/g dw) than that obtained from red grapes (24.1 mg GAE/g dw). These values were similar to those reported by Vatai et al. who registered a TPC for marc from red grapes between

17.3 and 20.2 mg GAE/g dw.[38] Differences in TPC between red and white marc are due to their different origins: white marc is produced during must production after crushing, while red marc is obtained during pressing after the alcoholic fermentation. Also, a high quantity of polyphenolic compounds of red grapes is dissolved in wine during wine-making.

Antioxidant capacity

Several methods have been developed to assess the *in vitro* antioxidant capacity of plant extracts. Relationships between assays were regulated by the method applied but also by the structure of antioxidants analyzed. Therefore, the use of at least two different analytical approaches to test the antioxidant capacity of specific substrates is recommended.[39] Buenger et al.[40] reported that the $DPPH^{\bullet}$ assay, followed by the $ABTS^{\bullet+}$ assay, yield the best results (based on reproducibility and sensitivity). These tests, involving chromogen compounds of a radical nature, are also the most common antioxidant capacity assays, used for their ease, speed and sensitivity.[41] In this study the $DPPH^{\bullet}$ and the $ABTS^{\bullet+}$ assays were thus selected to evaluate the antioxidant potential of extracts obtained from fruit and vegetable by-products (Table 3).

All products showed a scavenging activity against DPPH radical but significant differences were highlighted among extraction solvents. Generally, the aqueous acetonic extracts showed the highest antioxidant capacities and IP values range between 95.73% in pomegranate peel and 4.01% in "Piatlina" potato peel (Table 3). According to Kaur and Kapoor these values could be subdivided into three antioxidant activity groups: high (≥ 50%), moderate (20-50%) and low (< 20%).[42] Among the examined extracts, eight belonged to the former and included pomegranate peel (95.62%), hazelnut skin (92.90%), cornelian cherry seeds (77.44%), dog rose pulp (74.37%), marcs from red grape (65.98%), apple peel (63.44%), marcs from white grape (58.34 %) and cornelian cherry pulp (54.44%). The group with moderate activity was represented by dog rose seeds (45.40%), pomegranate pomace (24.91%), and "Viola" potato peel (20.34%). Finally, the other extracts could be included in the low anti-oxidant activity (< 20%) group. The total antioxidant

capacity of peel was generally significantly higher than pulp and pomace ($p< 0.05$).

The $ABTS^{\bullet+}$ method was used to confirm the results from the $DPPH^{\bullet}$ test since it is based on a similar antioxidant mechanism and the results are laid out in Table 3. The TEAC values ranged between 0.10 and 0.71 µM/g dw, showing the same trend reported for IP. The highest TEAC values (0.70 – 0.71 µM TE/g dw) were detected in hazelnut skin, pomegranate peel, apple peel, cornelian cherry pulp and seeds, white and red grape marcs, and dog rose pulp extracts. The lowest TEAC values were observed for "Piatlina" and "Desirée" potato peel extracts. Therefore, the results for TEAC tests are well in line with those of the $DPPH^{\bullet}$ assay.

For aqueous acetonic extracts, IP and TEAC values are directly correlated ($r=0.93$; $p<0.01$) according to their similar redox mechanism. These assays are also correlated with TPC ($r=0.78$ and $r=0.82$, $p<0.01$ respectively). There is no unanimous opinion about the relationship between the content of phenols and their antioxidant activity. Some authors observed close or very close correlations,[43,44] but this hypothesis was frequently discussed and opposed. Adopting the Folin-Ciocalteu method, various phenolic compounds have different responses to this assay, proportionally due to the number and positioning of hydroxyl groups. Since these structural features of phenols are also responsible for antioxidant activity, measurements of phenols in natural products may be related to this potential. In addition, the Folin-Ciocalteu assay mechanism is an oxidation/reduction reaction and, as such, can be considered another antioxidant method.[45]

Antibacterial activity

Employing the agar-well diffusion technique, the antibacterial activity of phenolic extracts against twelve food -related microorganisms was evaluated. The microorganisms selected belonged to pathogenic, spoilage or technologically important species, commonly found in foods. Only six products showed antimicrobial activity, and the highest values were highlighted for acetone and methanol extracts (Table 4). Higher antibacterial activity of acetone extracts was also reported by Negi and Jayaprakasha.[46] Two different extract concentrations were tested (10 and 20 mg of dry

extract/ml), but, as expected, higher activity was shown for the 20 mg/ml concentration. *Staphylococcus. marcescens*, *S. xylosus* and *Lb. sakei* were found to be the most resistant bacteria while *S. aureus* was the most sensitive. Pomegranate peel extracts were active against eleven bacterial species, and seed extracts against four. As reported by Negi and Jayaprakasha acetonic and methanolic extracts from pomegranate peel showed antimicrobial activity against *B. cereus*, *S. aureus*, *E. coli* and *P. aeuroginosa*.[46] As a large quantity of tannins were identified in pomegranate extracts,[47] Cowan suggested that the antibacterial properties of these extracts could be related to tannins and their activity to inactivate microbial adhesions, enzymes, and cell envelope transport proteins, and to modify the morphology of microorganisms.[48]

Apple peel and pomace extracts were active against eight and five microorganisms respectively. Fattouch et al.[49] reported that acetonic extracts from apple peel inhibited *S. aureus*, *B. cereus*, *P. aeuroginosa*, *E. coli*, and *Salmonella* spp. Peel extracts exhibited more antibacterial activity than pulp, according to their biochemical properties. Low activity was highlighted for cornelian cherry pulp extract, which showed activity for only four microorganisms, whereas dog rose pulp extract was active against an *E.coli* strain and *S. aureus*. Other extracts did not show any antimicrobial activity.

CONCLUSION

The ultrasonic extraction method with aqueous acetone mixture was the most effective for polyphenols from fruit and vegetable by-products. These extracts showed the highest value of antioxidant capacity. High values of polyphenolic content and antioxidant capacity were identified in pomegranate peel, hazelnut skin, cornelian cherry seed, marc and apple peel extracts. High antioxidant capacities were also shown by some minor fruits such as dog rose and cornelian cherry pulp. For some of these products antimicrobial activity was also observed, in particular against *S. aureus*, an important foodborne pathogen, and *P. fluorescens*, a spoilage microorganism. Further

studies are needed to evaluate the possible use of these fruit and vegetable by-products as natural food additives to increase their safety and nutritional value.

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Table captions

Table 1. Fruit and vegetable by-products examined

Table 2. Total phenolic content (TPC) in the plant extracts obtained with the three solvents. Data are means ± SD (n=3).

Table 3. Antioxidant capacity (AC) evaluated for extracts obtained with the three solvents. Data are means ± SD (*n*=3).

Table 4. Antibacterial activity of extracts obtained with the three solvents and evaluated by the agar- well diffusion assay. The concentration was 20 mg of dry extract/ml.

Table 1. Fruit and vegetable by-products examined.

Common name	**Scientific name**	**Families**	**Variety**	**Examined products**
Fruits				
Pomegranate	*Punica granatum*	*Lythraceae*	Dente di cavallo	Pomace – Peel
Apple	*Malus domestica*	*Rosaceae*	Grigia di Torriana	Pomace – Peel
Dog Rose	*Rosa canina*	*Rosaceae*	--	Pulp – Seeds
Cornelian cherry	*Cornus mas*	*Cornaceae*	--	Pulp – Seed
Hazelnut	*Corylus avellana*	*Corylaceae*	Round the Kind Trilobata	Skin
White grapes	*Vitis Vinifera*	*Vitaceae*	Chardonnay	Marc
Red grapes	*Vitis Vinifera*	*Vitaceae*	Nebbiolo	Marc
Vegetables				
Potato	*Solanum tuberosum*	*Solanaceae*	Viola	Pulp - Peel
Potato	*Solanum tuberosum*	*Solanaceae*	Desirée	Pulp - Peel
Potato	*Solanum tuberosum*	*Solanaceae*	Piatlina	Pulp - Peel
Leek	*Allium porrum*	*Liliaceae*	Monstrueux di Carentan	Non edible leaves

Table 2. Total phenolic content (TPC) in the plant extracts obtained with the three solvents. Data are means ± SD (n=3).

	TPC (mg GAE[†]/g dry weight)		
	Solvent A[††]	**Solvent B**[††]	**Solvent C**[††]
Hazelnut (skin)	124.6 ± 2.44 ^{h,A}	116.5 ± 5.25 ^{e,A}	166.3 ± 5.43 ^{f,B}
Pomegranate (peel)	197.1 ± 1.76 ^{g,B}	173.2 ± 3.54 ^{f,A}	212.3 ± 3.31 ^{g,C}
Apple (peel)	32.3 ± 1.14 ^{e,B}	20.1 ± 8.99 ^{c,A}	61.3 ± 4.65 ^{e,C}
"Viola" Potato (peel)	9.5 ± 0.29 ^{bc,A}	8.9 ± 1.40 ^{ab,A}	8.9 ± 0.08 ^{a,A}
"Desiree" Potato (peel)	5.9 ± 0.22 ^{ab,A}	7.4 ± 0.52 ^{a,B}	7.5 ± 0.08 ^{a,B}
"Piatlina" Potato (peel)	4.2 ± 0.09 ^{a,A}	4.2 ± 0.22 ^{a,A}	5.2 ± 0.07 ^{a,B}
Leek (leaves)	5.9 ± 0.08 ^{ab,A}	6.4 ± 0.50 ^{a,AB}	7.0 ± 0.31 ^{a,B}
Cornelian cherry (seed)	36.6 ± 5.51 ^{f,A}	33.3 ± 2.82 ^{d,A}	37.7 ± 3,32 ^{c,A}
Dog Rose (seeds)	21.2 ± 0.38 ^{d,B}	16.3 ± 0.31 ^{bc,A}	21.5 ± 0.33 ^{b,B}
White grape (marcs)	34.2 ± 1.25 ^{ef,A}	35.8 ± 9.76 ^{d,A}	50.5 ± 6.57 ^{d,B}
Red grape (marcs)	10.4 ± 1.68 ^{c,B}	6.6 ± 0.88 ^{a,A}	24.1 ± 0.75 ^{b,C}
Dog Rose (pulp)	19.9 ± 1.00 ^{e,B}	16.7 ± 0.64 ^{d,A}	85.5 ± 1.44 ^{f,C}
Cornelian cherry (pulp)	32.6 ± 1.32 ^{d,B}	26.8 ± 3.00 ^{e,A}	50.1 ± 2.77 ^{e,C}
Pomegranate (pomace)	9.9 ± 0.24 ^{c,B}	8.7 ± 0.45 ^{c,A}	13.2 ± 0.29 ^{d,C}
Apple (pomace)	8.9 ± 0.18 ^{c,A}	9.8 ± 1.55 ^{c,A}	10.4 ± 0.25 ^{c,A}
"Viola" Potato (pulp)	3.1 ± 0.20 ^{b,B}	2.4 ± 0.19 ^{ab,A}	2.6 ± 0.04 ^{ab,A}
"Desiree" Potato (pulp)	2.1 ± 0.42 ^{b,A}	4.2 ± 0.10 ^{b,B}	4.4 ± 1.04 ^{b,B}
"Piatlina" Potato (pulp)	0.5 ± 0.40 ^{a,A}	0.8 ± 0.43 ^{a,A}	1.6 ± 0.24 ^{a,B}

[†] Gallic acid equivalent.

[††] Solvent A = methanol (90%) in acidified water; solvent B = ethanol (80%) in water; solvent C = acetone (70%) in water.

$^{a-h}$ Values within column with the same letters are not significantly different at $p \leq 0.05$.

$^{A-C}$ Values within row with the same letters are not significantly different at $p \leq 0.05$.

Table 3. Antioxidant capacity (AC) evaluated for extracts obtained with the three solvents. Data are means ± SD (*n*=3).

	IP (%)			**TEAC** (μM TE[†]/g dry weight)		
	Solvent A[††]	**Solvent B**	**Solvent C**	**Solvent A**	**Solvent B**	**Solvent C**
Hazelnut (skin)	79.51 ± 5.18 ^{e,A}	86.58 ± 5.54 ^{h,AB}	92.90 ± 2.45 ^{h,B}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{e,A}
Pomegranate (peel)	95.73 ± 0.04 ^{f,A}	95.27 ± 0.57 ^{i,A}	95.62 ± 0.03 ^{h,A}	0.70 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{e,A}
Apple (peel)	49.33 ± 2.19 ^{d,B}	20.89 ± 2.26 ^{c,A}	63.44 ± 2.58 ^{ef,C}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{e,A}
"Viola" potato (peel)	23.50 ± 0.60 ^{b,B}	19.26 ± 0.80 ^{c,A}	20,34 ± 0.02 ^{c,A}	0.26 ± 0.01 ^{c,A}	0.26± 0.01 ^{c,A}	0.30 ± 0.02 ^{c,B}
"Desirée" potato (peel)	10.15 ± 0.61 ^{a,A}	12.83 ± 0.88 ^{b,A}	14.87 ± 0.93 ^{bc,A}	0.15 ± 0.01 ^{b,A}	0.19 ± 0.01 ^{b,B}	0.22 ± 0.01 ^{a,C}
"Piatlina" potato (peel)	8.42 ± 0.43 ^{a,A}	7.76 ± 0.61 ^{a,A}	10.17 ± 1.21 ^{ab,B}	0.13 ± 0.01 ^{a,A}	0.13 ± 0.01 ^{a,A}	0.20 ± 0.01 ^{a,B}
Leek (leaves)	5.19 ± 0.87 ^{a,A}	5.79 ± 0.92^{a,A}	5.89 ± 0.22^{a,A}	0.13 ± 0.01 ^{a,A}	0.19 ± 0.01 ^{b,B}	0.25 ± 0.04 ^{b,C}
Cornelian cherry (seed)	81.95 ± 7.66 ^{e,A}	76.44 ± 2.48 ^{g,A}	77.44 ± 6.86 ^{g,A}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{g,A}	0.71 ± 0.01 ^{e,A}
Dog Rose (seeds)	36.16 ± 2.22 ^{c,B}	31.74 ± 2.09 ^{d,A}	45.40 ± 0.05 ^{d,C}	0.53 ± 0,01^{d,B}	0.44 ± 0,01^{d,A}	0.62 ± 0,01^{d,C}
White grape (marcs)	41.68 ± 5.25 ^{c,A}	40.91 ± 4.99 ^{e,A}	58.34 ± 6.18 ^{e,B}	0.67 ± 0.01 ^{e,A}	0.67 ± 0.01 ^{e,A}	0.70 ± 0.01 ^{e,B}
Red grape (marcs)	52.96 ± 9.96 ^{d,A}	62.84 ± 3.21 ^{f,A}	65.98 ± 4.03 ^{f,A}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{f,A}	0.71 ± 0.01 ^{e,A}
Dog Rose (pulp)	72.48 ± 1.11 ^{d,A}	73.79 ± 2.34 ^{d,A}	74,37 ± 2.57 ^{d,A}	0.51 ± 0.02 ^{e,B}	0.40 ± 0.02 ^{c,A}	0.71 ± 0.01 ^{e,C}
Cornelian cherry (pulp)	59.03 ± 10.22 ^{c,B}	41.98 ± 3.51 ^{c,A}	54.44 ± 4.88 ^{c,A}	0.71 ± 0.01 ^{f,A}	0.69 ± 0.01 ^{d,A}	0.70 ± 0.01 ^{e,A}
Pomegranate (pomace)	26.19 ± 1.23 ^{b,B}	16.71 ± 3.08 ^{b,A}	24.91 ± 3.50 ^{b,B}	0.37 ± 0.02 ^{d,A}	0.37 ± 0.07 ^{c,A}	0.46 ± 0.03 ^{d,A}
Apple (pomace)	7.67 ± 0.28 ^{a,B}	5.55 ± 0.66 ^{a,A}	8.73 ± 1.06 ^{a,B}	0.26 ± 0.03 ^{c,A}	0.28 ± 0.01 ^{b,AB}	0.32 ± 0.02 ^{c,B}
"Viola" potato (pulp)	8.48 ± 0.17 ^{a,C}	4.66 ± 0.24 ^{a,A}	6.93 ± 0.03 ^{a,B}	0.11 ± 0.01 ^{b,A}	0.14 ± 0.01 ^{a,B}	0.17 ± 0.01 ^{b,C}
"Desiree" potato (pulp)	4.88 ± 0.10 ^{a,A}	5.58 ± 1.29 ^{a,A}	5.81 ± 0.27 ^{a,A}	0.07 ± 0.01 ^{a,A}	0.11 ± 0.01 ^{a,B}	0.12 ± 0.01 ^{a,B}
"Piatlina" potato (pulp)	4.75 ± 0.08 ^{a,A}	4.01 ± 0.35 ^{a,B}	4.01 ± 0.13 ^{a,A}	0.08 ± 0.01 ^{a,A}	0.15 ± 0.07 ^{a,A}	0.10 ± 0.01 ^{a,A}

[†] Trolox Equivalent

[††] Solvent A = methanol (90%) in acidified water; solvent B = ethanol (80%) in water; solvent C = acetone (70%) in water.

[a-h] Values within column by the same letters are not significantly different at $p \leq 0.05$.

[A-C] Values within row by the same letters are not significantly different at $p \leq 0.05$.

Table 4. Antibacterial activity of extracts obtained with the three solvents and evaluated by the agar-well diffusion assay. The concentration was 20 mg of dry extract/ml.

	Pomegranate (peel)			**Pomegranate (pomace)**			**Apple (peel)**			**Apple (pomace)**			**Cornelian cherry (pulp)**			**Dog rose (pulp)**		
Solvent[†]	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Salmonella Enteritidis O:103	+[a]	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Pseudomonas fluorescens	++	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-
Escherichia coli ATCC 35150	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli DH5α	+	-	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-	+
Serratia marcescens	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 10527	+	+	++	+	+	+	-	+	+	-	-	+	-	-	+	+	+	+
Listeria monocytogenes NCTC 10527	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
Bacillus cereus DSM 350	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Lactococcus lactis DSM 4366	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+
Lactobacillus sakei DSMZ 6333	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Staphylococcus xylosus	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[†] Solvent A = methanol (90%) in acidified water; solvent B = ethanol (80%) in water; solvent C = acetone (70%) in water.

[a] -: inhibition zone ≤1 mm, +: 1 mm < inhibition zone ≤ 3mm, ++: inhibition zone >3 mm